Consortium News—

Newsletter of the SERDP Biotreatment Research Consortium

US Army Corps
of Engineers
Waterways Experiment
Station

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Research initiatives by the Consortium

The Federal Integrated Biotreatment Research Consortium, referred to as the "Consortium," has begun several research tasks to develop innovative biotreatment processes. Consortium activities are aligned into five primary research thrust areas: biotreatment of explosives, polychlorinated biphenyls (PCBs), heavy molecular-weight polycyclic aromatic hydrocarbons (hPAHs), chlorinated solvents, and the development of specialty bioreactors. This issue of *Consortium News* is devoted primarily to ongoing research efforts. Additional items include a "Technology Profile" on composting and Consortium news items.

Research thrust areas—overview

The Consortium's research thrust areas focus on U.S. Department of Defense (DoD) cleanup problems. Some of the technologies being developed also have a high potential for use in remediating non-DoD sites, such as Superfund Sites and contaminated sediments. A brief overview of each major work unit is given below. Requests for more detailed information on any work unit can be directed to the Consortium Co-Directors or to the point of contact (POC) listed for each work unit.

Consortium points of contact

Consortium Director: Dr. Kurt Preston, U.S. Army Engineer Waterways Experiment Station

Co-Director for Engineering: Mr. Jeffrey W. Talley, U.S. Army Engineer Waterways Experiment Station

Co-Director for Science: Dr. Herb Fredrickson, U.S. Army Engineer Waterways Experiment Station

Design Implementation: Ms. Chris Correale, U.S. Army Engineer District, Baltimore

Explosives Research: Dr. Charlene Mello, U.S. Army Natick Research, Development and Engineering Center

Chlorinated Solvents Research: Dr. Herb Fredrickson, U.S. Army Engineer Waterways Experiment Station

PAH Research: Dr. Sabine Apitz, U.S. Navy

PCB Research: Dr. Jim Tiedje, Michigan State University

Explosives

Explosives compounds are a major pollution problem for the DoD. Contamination at DoD installations has occurred as a result of manufacturing, packing, and disposal operations. Current research efforts by the Consortium on explosives are summarized below.

- **Phytoremediation.** The U.S. Environmental Protection Agency's (EPA) Environmental

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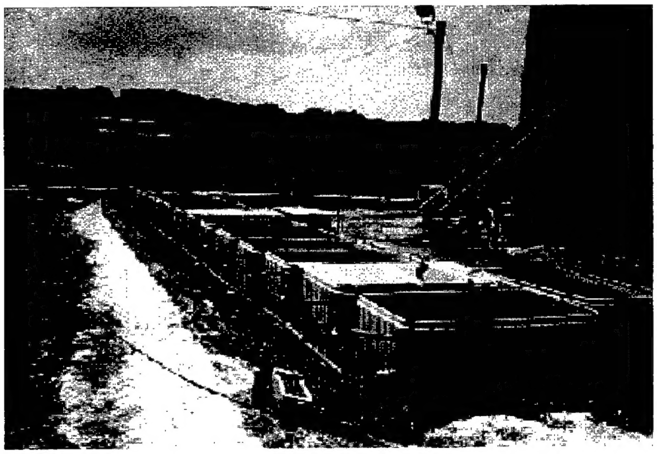


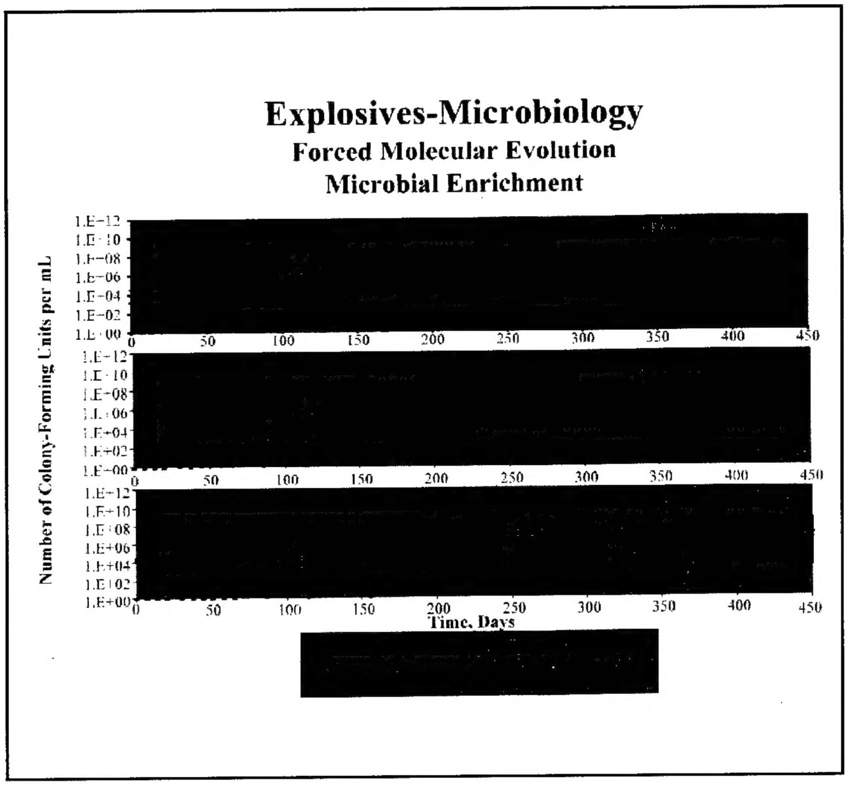
Phytoremediation field study, Volunteer Army Ammunition Plant

Restoration Laboratory in Athens, GA, is developing plant-based remediation techniques (phytoremediation) for treatment of contaminated groundwater. These efforts are being conducted with the USAE Waterways Experiment Station (WES). Three bench studies are under way to evaluate the capability of plants to remove the explosives components of groundwaters from Iowa Army Ammunition Plant (AAP), Milan AAP, and Volunteer AAP. A pilot study is scheduled at Volunteer AAP during summer 1996. The U.S. Army Environmental Center (AEC), as lead agency, along with the USEPA, the Tennessee Valley Authority (TVA), and WES, will demonstrate phytoremediation at Milan AAP. *POCs are Dr. Steve McCutcheon, USEPA, (706-546-3301) and Mr. Jerry Miller, WES, (601-634-3931).*

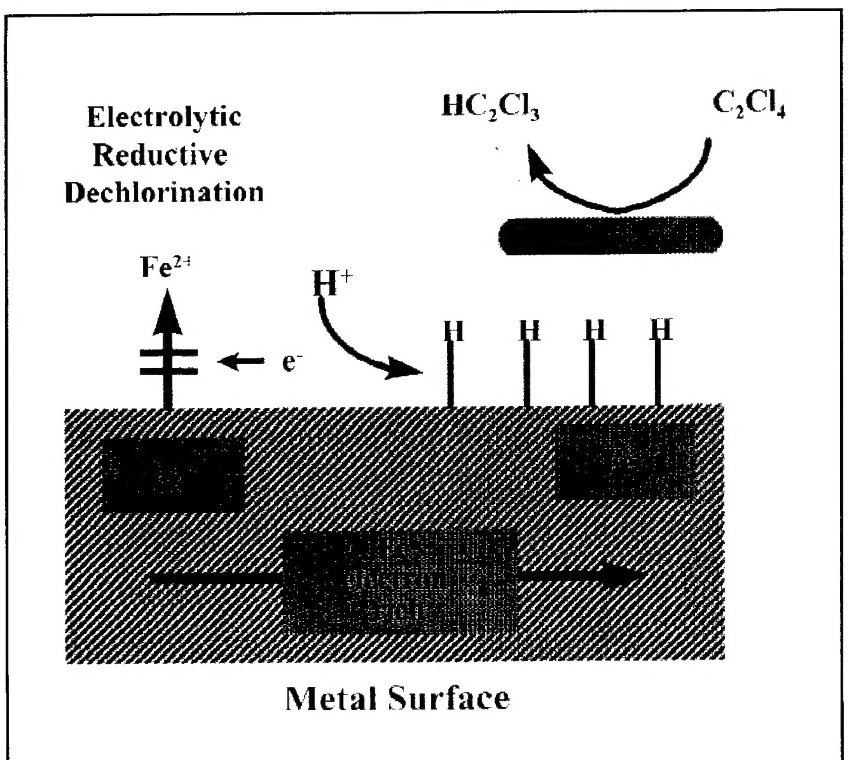
- **Evaluation of REDOX conditions.** Past research efforts on the biotreatment of explosives-contaminated soils have indicated that both aerobic and anaerobic techniques are viable options. Unfortunately, these individual efforts were not performed on the same soils under the same experimental conditions. WES is currently evaluating both aerobic and anaerobic biochemical mechanisms using two bioreactor designs: bioslurry and biocells. This approach will allow researchers to directly compare aerobic and anaerobic systems and to evaluate how the reactor environment impacts performance. *POC is MAJ Steve Harvey, WES, (601-634-4843).*
- **Surfactant enhancement.** WES is also evaluating the merits of amending bioreactors treating explosives- contaminated soils with non-ionic surfactants. Results indicate that this approach can reduce soil residence times in the reactor by as much as 50 percent. Surfactant dosages ranging from 0.5 to 5 percent are being evaluated for their relative impact on explosives removal. *POC is MAJ Steve Harvey, WES, (601-634-4843).*
- **Oxidative pre- and posttreatment.** The concept of combining chemical oxidation with biological treatment has received significant attention over the past 5 years. This is an extremely effective combination because oxidation processes typically excel in breaking down large organic molecules, which are usually difficult to biodegrade. Oxidation processes are rate-limited in terms of further breaking down the straight-chained products. Biotreatment is the opposite. Microorganisms often have difficulty breaking down large molecules, especially those containing aromatic rings. However, microorganisms thrive on smaller, straight-chained chemicals such as aldehydes. WES is currently evaluating the combined use of oxidation processes with biotreatment. The use of oxidation as pre-, post-, or stand-alone technology is being tested with 5-liter slurry reactors. *POC is Ms. Beth Fleming, WES, (601-634-3943).*
- **Microbial inoculum development/pathway definition.** The U.S. Army's Natick Materials Research Laboratory, the U.S. Air Force's Armstrong Laboratory, and WES are evaluating various gene-manipulation techniques for developing improved bacterial species with increased explosives biodegradation potential. To date, a proven microorganism to mineralize explosive compounds has not been demonstrated. It is hoped that this effort may one day develop a single organism (isolate) or group of organisms (consortia) with this capability. Also, this team of researchers is evaluating the degradative pathways used by the engineered microorganisms and some natural cultures to degrade the explosives. *POCs are Dr. Charlene Mello, Natick Laboratory, (508-733-5825); Dr. Jim Spain, USAF-Armstrong Laboratory, (909-283-6233); and Dr. Douglas Gunnison, WES, (601-634-3873).*

Chlorinated solvents

Chlorinated solvents represent one of the most prevalent contaminant groups found at DoD installations. Their usage was quite widespread because of their very efficient degreasing and solvent properties. Ongoing research efforts by the Consortium are described below.



Forced molecular evolution research is seeking ways to degrade explosives



Electrolytic techniques enhance the mechanisms of bioactive treatment

- **Anaerobic dechlorination.** Researchers at Michigan State University are evaluating the ability of anaerobic consortia to degrade chlorinated solvents. This approach includes evaluation of key physiological characteristics of organisms that have a high anaerobic dehalogenation activity. Isolation of microorganisms from various environmental media is under way to find suitable microorganisms and those ecological systems that best support them. *POC is Dr. James Tiedje, Michigan State University, (517-353-9021).*
- **Electrolytic enhancements.** The USEPA-Robert S. Kerr Laboratory (EPA-RSK), Ada, Oklahoma, is also evaluating anaerobes and their ability to dechlorinate solvents. The researchers are enhancing these mechanisms by incorporating electrolytic techniques for adding hydrogen. Electrolysis provides hydrogen, which appears to be an excellent electron acceptor. The electrolytic/anaerobic biodegradation approach is being developed for use within bioactive treatment wells. *POC is Dr. John Wilson, EPA-RSK, (405-436-8532).*
- **Co-metabolic, aerobic degradation.** The use of aerobic microorganisms that require co-metabolites to stimulate dechlorination activity is under investigation by WES. These efforts are being performed under collaboration with Envirogen, Inc., a leader in the commercialization of biotreatment systems. Particular emphasis is being placed on the ecological requirements for the long-term maintenance of key aerobic microorganisms. This effort also includes development of kinetic models and the evaluation of methanotrophic communities (aerobes that use methane as a

co-metabolite) for bioventing of solvents-contaminated vadose zones. *POC is Dr. Herb Frederickson, WES, (601-634-3716).*

- **Phytoremediation.** The EPA-Athens Laboratory is also assessing the ability of plants to treat solvents-contaminated groundwaters. The concept involves pumping of the groundwater into wetlands containing plant species with high levels of haloreductases. The basis for this effort is the positive results they have been obtained using haloreductases for dechlorination of PCBs. *POC is Dr. Lee Wolfe, EPA, (706-546-3429).*

Polychlorinated biphenyls

PCBs have been widely used as insulating fluids and as a component of hydraulic and machining oils. Typically, they are found at small sites within DoD installations and are a major contaminant for sediments. Current activities within the Consortium regarding PCBs are summarized below.

- **Surfactant enhancement.** Researchers from both the University of Michigan and Georgia Tech are experimenting with various surfactants for their relative abilities to enhance the biodegradation rate of these compounds due to increased bioavailability. All of the candidate surfactants are biodegradable and should not pose any environmental concern with their usage within the environment. *POC is Dr. Walt Weber, University of Michigan, (313-763-2274).*
- **Dehalogenation activity.** Various anaerobic microbial consortia are being evaluated by Michigan State University for dechlorination of highly chlorinated PCBs. Various co-metabolites are under investigation as to the impact of co-metabolite species on the rate of aerobic biodegradation of the PCBs that have undergone dechlorination by the anaerobes. *POC for this effort is Dr. Jim Tiedje, Michigan State University, (517-353-9021).*
- **Isolation of consortia.** WES is isolating various consortia from samples of sediments and soils for their ability to aerobically and anaerobically both degrade and dechlorinate PCBs. A series of experiments are under way to selectively culture key consortia organisms with the highest degree of metabolic activity to PCBs with varying levels of dechlorination. *POC is Dr. Herb Frederickson, WES, (601-634-3716).*

Heavy polycyclic aromatic hydrocarbons

PAHs are a component of many petroleum-based products, including heating oils and vehicular fuels, all of which were and are commonly used by the DoD. Unfortunately, the heavier the PAHs (that is, the higher the number of aromatic rings), the higher the environmental concern due to the potential carcinogenicity of the PAH.

- **Composting.** The Department of Civil Engineering at Howard University is evaluating the potential for using composting systems to degrade heavy PAHs within soil matrices. Benzo(a)pyrene is the test compound for this effort (refer to "Technology Profile," page 5). *POC is Dr. Jim Johnson, Howard University, (202-806-6565).*
- **Cascading bioslurry.** The U.S. Navy-NRAD Laboratory is using bioslurry systems configured as cascading reactors to accelerate biodegradation of heavy PAHs. The approach involves designing a series of three bioslurry reactors in which soil-slurries treated in the previous reactors are periodically added to the next reactor. The concept is that differing microbial consortia will be sustained in each bioreactor based on the types of PAHs remaining after treatment within the previous reactor. Usually, the lighter, easier to degrade PAHs are removed early into the cascade series, requiring that different, more aggressive microorganims be maintained in the bioslurry reactors downstream. *POC is Dr. Sabine Apitz, NRAD, (619-553-6305).*
- **Natural bioemulsifiers and biosurfactants.** The U.S. Navy-Surface Warfare Center is developing microbial populations with capabilities of producing high levels of bioemulsifiers. Originally, this approach was evaluated by the Navy for use in the degradation of enemy asphalt surfaces (airfields and service roads). Research indicated that these organisms were too slow to destroy asphalt surfaces within required time frames. However, it is hoped that these microorganisms will produce enough bioemulsifiers to increase the rate of PAH degradation via increased bioavailability. *POC is Dr. Joanne Jones-Meehan, U.S. Naval Research Laboratory, (202-404-6361).*
- **Isolate and consortium development.** WES is focusing on isolation and rapid deployment of consortia with high activities toward heavy PAHs. It is hoped that, by rapidly isolating PAH degraders native to the site, these consortia can be grown to seeding levels and then reintroduced to the contaminated soils. The use of surfactants with the

cultured consortia is being evaluated over nonamended bioreactor systems. *POC is Dr. Douglas Gunnison, WES, (601-634-3873).*

- **Seeding incubators.** The use of seed incubators placed onsite to continually bombard the targeted treatment zone with active isolates during *in situ* biotreatment is under investigation by the University of Michigan. The idea under development is that, all too often, specialty bacteria added to *in situ* systems are rapidly outcompeted by the native microorganisms already onsite. Unfortunately, the native organisms likely do not have the degradation potential provided by the seeded specialty organisms. *POC is Dr. Walt Weber, University of Michigan, (313-763-2274).*

Bioreactor development

Bioreactors are the reactor support systems to biochemical reactions mediated by microorganisms. Current bioreactor designs generally are not very conducive to economically nor technically supporting the targeted microbial populations. It is anticipated that the efforts discussed below will assist in the further development of innovative bioreactor designs that may be implemented by design engineers during site remediation.

- **Process gas recirculation.** WES is evaluating the feasibility of using process gas recirculation to aerobically treat solvent-contaminated soils. Process gas recirculation allows for the highly volatile solvents to be oxygenated within the slurries without stripping the solvents or co-metabolites (methane, toluene, or phenol) into the atmosphere because the process gases are continuously recirculated within a closed loop. A microprocessor unit monitors oxygen, carbon dioxide, and system pressure within the recirculation loop. Oxygen is added as needed to maintain a process gas composition of at least 19 percent oxygen. Carbon dioxide is removed using a molecular sieve to prevent lowering of the system pH or excessive buildup of carbon dioxide. *POC is Mr. Jeff Talley, WES, (601-634-2856).*
- **Biocells.** Biocells are bioreactors contained in 76-liter (20-gallon) drums filled with contaminated soils and operated as aboveground bioventing systems. This technology, featured in the previous issue of *Consortium News*, was recently evaluated using a 7.6-cubic meter (10-cubic yard) system at Port Hueneme, California, in collaboration with the U.S. Navy. Port Hueneme is a SERDP National Test Site. Results are under evaluation, but preliminary observations indicate that this technology can be applied at costs significantly lower than bioslurry and similar to those reported for landfarming. *POC is Mr. Jeff Talley, WES, (601-634-2856).*

Technology profile

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous chemicals in nature and are made up of fused benzene rings in linear, angular, or cluster arrangements. Eleven of the forty PAHs are listed as strongly carcinogenic, cytotoxic, or mutagenic and often are listed as carcinogenic or mutagenic. Typically, the higher the number of benzene rings, the greater the carcinogenicity of the compound. Because of health concerns, sixteen PAHs are listed by EPA as priority pollutants, and research is under way in various institutions to find viable technologies to remediate PAH-contaminated soils.

A most promising method of detoxifying or destroying organic waste is composting technology. Composting technology is primarily a microbial process under controlled conditions to convert organic waste into partially stabilized (oxidized) residue.

The cost-effectiveness of composting and its effectiveness to convert hazardous substances into innocuous end products make this technology superior to other methods of mitigating toxins. The intensity of microbial activity in a composting matrix accelerates the process of biodegradation, thus reducing the time required for waste reduction.

The Department of Civil Engineering, Howard University, is in the forefront of developing and implementing composting technology for bioremediation of organic wastes. Previous work involved the development and use of batch and continuously aerated composters for biodegradation of pyrene and trinitrobenzoic acid (TNBA).

Using a factorial design approach, parametric models are developed to relate composting process performance to pH, moisture content, and temperature. In a batch composter maintained at 65 °C, about 70 percent of pyrene disappeared in 26 days.

Both the temperature and pH influenced biodegradation. While decomposition is enhanced at optimum temperature, the speed at which such temperature is reached is greatly affected by pH. While composting, the disappearance of pyrene and $^{14}CO_2$ evolution were highest within the thermophilic temperature range. Maximum biological activity was observed at or around neutral pH.

Recent research focus is on degradation of highly recalcitrant and high molecular weight PAHs. Though microbial degradation pathways of lower PAHs are well documented, little is known about the reactions involved in the bacterial degradation of high molecular weight PAHs such as pentacyclic aromatic hydrocarbons. As the number of benzene rings in a PAH increases, it becomes harder for the microbes to break the bonds and degrade the compound. The model compound used in the present study is benzo(a)pyrene (BaP), a five-ring PAH. Microbial mineralization of this compound under controlled conditions such as composting remains an unproven concept and a challenge. Composting of BaP-spiked soil in batch composter after inoculating with bacteria has little impact on the disappearance of BaP.

Since summer 1995, funding through the Consortium has enabled the Department of Civil Engineering to initiate a multifaceted and comprehensive investigation to develop a viable composting technology strategy for the biodegradation of hPAHs. Howard University is one of the participants in this multiagency research consortium involved in PAH bioremediation studies. Other prominent agencies actively working on well-defined tasks of this project include the U.S. Navy, EPA, and WES. The thrust areas of research undertaken by the Department of Civil Engineering include development of batch (Figure 1) and continuously aerated composters, bioaugmentation, use of cosubstrates and surfactants, and manipulation of the physicochemical properties of soil to enhance bacterial growth and subsequent degradation of BaP.

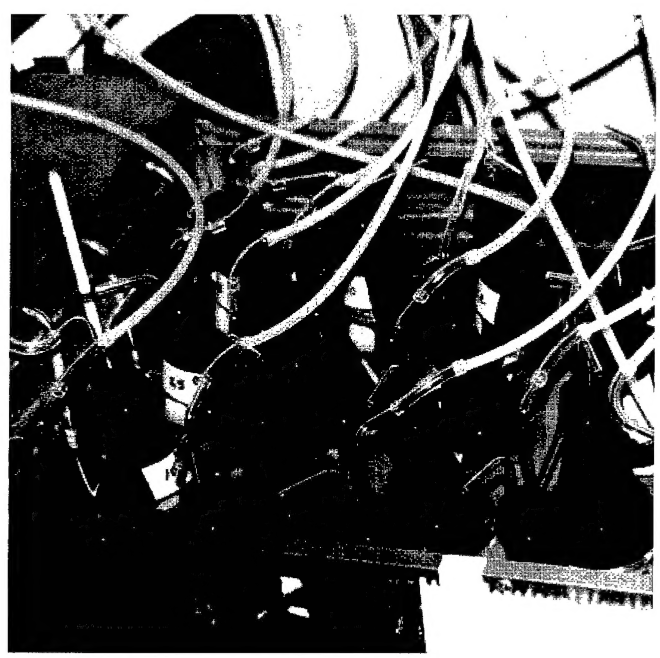


Figure 1. Aerated batch composter. The bioreactor flasks contain BaP-spiked soil and microbial inoculum maintained at a specific temperature and moisture level.

Other news

New Consortium Director

Dr. Mark Zappi, former Consortium Director, assumed a faculty position with the Department of Chemical Engineering, Mississippi State University, in March 1996.

The new Director, Dr. Kurt Preston of the Waterways Experiment Station, looks forward to working with the Consortium members on his return to civilian duty. On May 17, he and other members of the 412th Engineer Command were activated to support Operation Joint Endeavor, the North Atlantic Treaty Organization's peacekeeping effort in Bosnia. Dr. Preston's talents are currently helping U.S. Forces to comply with applicable environmental laws and regulations. Dr. Preston is expected to return to WES in January 1997. The Consortium members extend their best wishes to Dr. (Major) Preston for a successful mission and a safe return.

Consortium Co-Directors

Further information about the functions and activities of the Consortium can be obtained by contacting the Consortium Co-Directors. Mr. Jeff Talley, Co-Director for Engineering, can be reached at 601-634-2856, e-mail talleyj@ex1.wes.army.mil. Dr. Herb Fredrickson, Co-Director for Science, can be reached at 601-634-3716, e-mail fredrih@ex1.wes. army.mil.

Successful 1996 Consortium Member Meeting

The 1996 Consortium Member Meeting was held during April 1996. The USEPA's Athens Laboratory hosted the meeting. A summary of the meeting will be featured in the next issue of the *Consortium News.*

Explosives Bioremediation Planning Workshop

The SERDP Consortium, along with the USEPA's South and Southwest Hazardous Substance Research Center and the U.S. Army Environmental Center, sponsored a meeting on the future directions of explosives bioremediation developments and utilization. The meeting, hosted by Georgia Institute of Technology, brought together experts from various government and private groups having interests in the development of biotechnologies for explosives remediation. Several suggestions were made pertaining to defining successful development, buy-in by regulatory groups, impediments to technology use, and future planning meetings. A report detailing the intent and results of the meeting will be available in the near future.

Current Consortium Membership

Name	Fax Number	Organization
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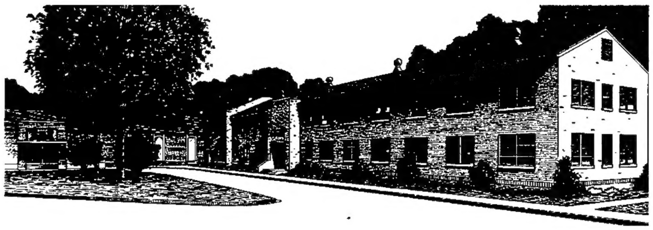

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